

## CLAIMS

We claim:

1. A recombinant expression construct comprising at least one regulated promoter operably linked to a first nucleic acid encoding an adenine nucleotide translocator polypeptide.
2. The expression construct of claim 1 further comprising at least one additional nucleic acid sequence that regulates transcription.
3. The expression construct of claim 2 wherein the additional nucleic acid sequence that regulates transcription encodes a repressor of said regulated promoter.
4. The expression construct of claim 1 wherein the adenine nucleotide translocator polypeptide comprises a human adenine nucleotide translocator polypeptide.
5. The expression construct of claim 4 wherein the human adenine nucleotide translocator polypeptide is ANT1.
6. The expression construct of claim 4 wherein the human adenine nucleotide translocator polypeptide is ANT2.
7. The expression construct of claim 4 wherein the human adenine nucleotide translocator polypeptide is ANT3.
8. An expression construct according to claim 1 wherein the adenine nucleotide translocator polypeptide is expressed as a fusion protein with a polypeptide product of a second nucleic acid sequence.
9. The expression construct of claim 8 wherein the polypeptide product of said second nucleic acid sequence is an enzyme.

10. The expression construct of claim 8 wherein said fusion protein localizes to membranes.

11. The expression construct of claim 10 wherein said membranes are mitochondrial membranes.

12. An expression construct according to claim 1 wherein the adenine nucleotide translocator polypeptide is expressed as a fusion protein with at least one product of a second nucleic acid sequence encoding a polypeptide cleavable by a protease, said adenine nucleotide translocator polypeptide being separable from the fusion protein by cleavage with the protease.

13. A host cell comprising a recombinant expression construct according to claim 1.

14. A host cell according to claim 13 wherein the host cell is a prokaryotic cell.

15. A host cell according to claim 13 wherein the host cell is a eukaryotic cell.

16. The host cell of claim 15 wherein the eukaryotic cell is selected from the group consisting of a yeast cell, an insect cell and a mammalian cell.

17. The host cell of claim 16 wherein the insect cell is selected from the group consisting of an Sf9 cell and a *Trichoplusia ni* cell.

18. A host cell according to claim 13 that lacks at least one isoform of an endogenous adenine nucleotide translocator.

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19. A host cell according to claim 13 in which expression of at least one gene encoding an endogenous adenine nucleotide translocator isoform is substantially impaired.
  20. A recombinant expression construct comprising at least one promoter operably linked to a nucleic acid molecule comprising a first nucleic acid sequence and a second nucleic acid sequence, said first nucleic acid sequence encoding an animal adenine nucleotide translocator polypeptide wherein the adenine nucleotide translocator polypeptide is expressed as a fusion protein with a polypeptide product of said second nucleic acid sequence.
  21. The expression construct of claim 20 wherein the polypeptide product of said second nucleic acid sequence is an enzyme.
  22. The expression construct of claim 20 wherein said fusion protein localizes to membranes.
  23. The expression construct of claim 22 wherein said membranes are mitochondrial membranes.
  24. The expression construct of claim 20 further comprising at least one additional nucleic acid sequence that regulates transcription.
  25. The expression construct of claim 24 wherein the additional nucleic acid sequence that regulates transcription encodes a repressor of said promoter.

26. The expression construct of claim 20 wherein the adenine nucleotide translocator polypeptide comprises a human adenine nucleotide translocator polypeptide.

27. The expression construct of claim 26 wherein the human adenine nucleotide translocator polypeptide is ANT1.

28. The expression construct of claim 26 wherein the human adenine nucleotide translocator polypeptide is ANT2.

29. The expression construct of claim 26 wherein the human adenine nucleotide translocator polypeptide is ANT3.

30. An expression construct according to claim 20 wherein the adenine nucleotide translocator polypeptide is expressed as a fusion protein with at least one product of a second nucleic acid sequence encoding a polypeptide cleavable by a protease, said adenine nucleotide translocator polypeptide being separable from the fusion protein by cleavage with the protease.

31. A host cell comprising a recombinant expression construct according to claim 20.

32. A host cell according to claim 31 wherein the host cell is a prokaryotic cell.

33. A host cell according to claim 31 wherein the host cell is a eukaryotic cell.

34. The host cell of claim 33 wherein the eukaryotic cell is selected from the group consisting of a yeast cell, an insect cell and a mammalian cell.

35. The host cell of claim 34 wherein the insect cell is selected from the group consisting of an Sf9 cell and a *Trichoplusia ni* cell.

36. A host cell according to claim 20 that lacks at least one isoform of an endogenous adenine nucleotide translocator.

37. A host cell according to claim 20 in which expression of at least one gene encoding an endogenous adenine nucleotide translocator isoform is substantially impaired.

38. A recombinant expression construct according to either claim 1 or claim 20 wherein the expression construct is a recombinant viral expression construct.

39. A method of producing a recombinant adenine nucleotide translocator polypeptide, comprising:

culturing a host cell comprising a recombinant expression construct comprising at least one regulated promoter operably linked to a first nucleic acid encoding an adenine nucleotide translocator polypeptide.

40. A method of producing a recombinant adenine nucleotide translocator polypeptide, comprising:

culturing a host cell comprising a recombinant expression construct comprising at least one promoter operably linked to a nucleic acid molecule comprising a first nucleic acid sequence and a second nucleic acid sequence, said first nucleic acid sequence encoding an animal adenine nucleotide translocator polypeptide wherein the

adenine nucleotide translocator polypeptide is expressed as a fusion protein with a polypeptide product of said second nucleic acid sequence.

41. A method of producing a recombinant adenine nucleotide translocator polypeptide, comprising:

culturing a host cell infected with the recombinant viral expression construct of claim 38.

42. An ANT polypeptide produced by the method of any one of claims  
39-41.

43. An isolated human adenine nucleotide translocator polypeptide.

44. The isolated polypeptide of claim 43 wherein the human adenine nucleotide translocator polypeptide is recombinant ANT1 or a variant or fragment thereof.

45. The isolated polypeptide of claim 43 wherein the human adenine nucleotide translocator polypeptide is recombinant ANT2 or a variant or fragment thereof.

46. The isolated polypeptide of claim 43 wherein the human adenine nucleotide translocator polypeptide is recombinant ANT3 or a variant or fragment thereof.

47. An isolated human adenine nucleotide translocator fusion protein comprising an adenine translocator polypeptide fused to at least one additional polypeptide sequence.

48. The fusion protein of claim 47 wherein said one additional polypeptide sequence is an enzyme sequence or a variant or fragment thereof.

49. The fusion protein of claim 47 wherein said fusion protein localizes to membranes.
50. The fusion protein of claim 49 wherein said membranes are mitochondrial membranes.
51. An isolated human adenine nucleotide translocator fusion protein comprising an adenine translocator polypeptide fused to at least one additional polypeptide sequence cleavable by a protease, said adenine nucleotide translocator polypeptide being separable from the fusion protein by cleavage with the protease.
52. An isolated adenine nucleotide translocator fusion protein comprising a first polypeptide that is an animal adenine translocator polypeptide fused to at least one additional polypeptide sequence.
53. The fusion protein of claim 52 wherein said one additional polypeptide sequence is an enzyme sequence or a variant or fragment thereof.
54. A fusion protein according to claim 52 that localizes to membranes.
55. A fusion protein according to claim 54 wherein said membranes are mitochondrial membranes.
56. An isolated recombinant animal adenine nucleotide translocator fusion protein comprising an adenine translocator polypeptide fused to at least one additional polypeptide sequence cleavable by a protease, said adenine nucleotide translocator polypeptide being separable from the fusion protein by cleavage with the protease.

57. The fusion protein of either claim 47 or claim 52 wherein the additional polypeptide sequence is a polypeptide having affinity for a ligand.

58. A method for determining the presence of an ANT polypeptide in a biological sample comprising:

contacting a biological sample suspected of containing an ANT polypeptide with an ANT ligand under conditions and for a time sufficient to allow binding of the ANT ligand to an ANT polypeptide; and

detecting the binding of the ANT ligand to an ANT polypeptide, and therefrom determining the presence of an ANT polypeptide in said biological sample.

59. The method of claim 58 wherein the adenine nucleotide translocator polypeptide comprises a human adenine nucleotide translocator polypeptide.

60. The method of claim 59 wherein the human adenine nucleotide translocator polypeptide is ANT1.

61. The method of claim 59 wherein the human adenine nucleotide translocator polypeptide is ANT2.

62. The method of claim 59 wherein the human adenine nucleotide translocator polypeptide is ANT3.

63. The method of claim 58 wherein the ANT ligand comprises atractyloside substituted at 6' hydroxyl to form an atractyloside derivative.

64. The method of claim 63 wherein the atractyloside is detectably substituted at the 6' hydroxyl to form a detectable atractyloside derivative.

65. The method of claim 64 wherein the detectable atractyloside derivative comprises a radiolabeled substituent.

66. The method of claim 65 wherein the radiolabeled substituent is selected from the group consisting of  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^3\text{H}$ ,  $^{14}\text{C}$  and  $^{35}\text{S}$ .

67. The method of claim 64 wherein the detectable atractyloside derivative comprises a fluorescent substituent.

68. The method of claim 67 wherein the ANT ligand further comprises a  $\text{Eu}^{3+}$  atom complexed to the atractyloside derivative.

69. The method of claim 64 wherein the detectable atractyloside derivative comprises covalently bound biotin.

70. The method of claim 63 wherein the atractyloside molecule is substituted at 6' hydroxyl with an amine or an amine containing functionality to form an amine modified atractyloside derivative.

71. The method of any one of claims 63-70 wherein the atractyloside molecule is a carboxyatractyloside molecule that is substituted at 6' hydroxyl to form an atractyloside derivative that is a carboxyatractyloside derivative.

72. A method for isolating ANT from a biological sample, comprising:  
contacting a biological sample suspected of containing an ANT polypeptide with an ANT ligand under conditions and for a time sufficient to allow binding of the ANT ligand to an ANT polypeptide; and  
recovering the ANT polypeptide, and thereby isolating ANT from a biological sample.

73. The method of claim 72 wherein the ANT ligand is covalently bound to a solid phase.

74. The method of claim 72 wherein the ANT ligand is non-covalently bound to a solid phase.

75. A method for identifying an agent that binds to an ANT polypeptide, comprising:

contacting a candidate agent with a host cell expressing at least one recombinant ANT polypeptide under conditions and for a time sufficient to permit binding of the agent to said recombinant ANT polypeptide; and

detecting binding of said agent to the recombinant ANT.

76. The method of claim 75 wherein the host cell is a prokaryotic cell.

77. The method of claim 76 wherein the prokaryotic cell is an *E. coli* cell.

78. The method of claim 75 wherein the host cell is a eukaryotic cell.

79. The method of claim 78 wherein the eukaryotic cell is selected from the group consisting of a yeast cell, an insect cell and a mammalian cell.

80. The method of claim 79 wherein the insect cell is selected from the group consisting of an Sf9 cell and a *Trichoplusia ni* cell.

81. The method of any one of claims 75-80 wherein the host cell lacks at least one isoform of an endogenous adenine nucleotide translocator.

82. The method of any one of claims 75-80 wherein host cell expression of at least one gene encoding an endogenous adenine nucleotide translocator isoform is substantially impaired.

83. A method for identifying an agent that binds to an ANT polypeptide, comprising:

contacting a candidate agent with a biological sample containing at least one recombinant ANT polypeptide under conditions and for a time sufficient to permit binding of the agent to said ANT polypeptide; and

detecting binding of said agent to the recombinant ANT polypeptide.

84. A method for identifying an agent that interacts with an ANT polypeptide comprising:

contacting a biological sample containing recombinant ANT with a detectable ANT ligand in the presence of a candidate agent; and

comparing binding of the detectable ANT ligand to recombinant ANT in the absence of said agent to binding of the detectable ANT ligand to recombinant ANT in the presence of said agent, and therefrom identifying an agent that interacts with an ANT polypeptide.

85. An ANT ligand comprising atractyloside substituted at the 6' hydroxyl to form an atractyloside derivative.

86. The ANT ligand of claim 85 wherein the atractyloside is detectably substituted at the 6' hydroxyl to form a detectable atractyloside derivative.

87. The ANT ligand of claim 86 wherein the detectable atractyloside derivative comprises a radiolabeled substituent.

88. The ANT ligand of claim 87 wherein the radiolabeled substituent is selected from the group consisting of  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^3\text{H}$ ,  $^{14}\text{C}$  and  $^{35}\text{S}$ .

89. The ANT ligand of claim 86 wherein the detectable atractyloside derivative comprises a fluorescent substituent.

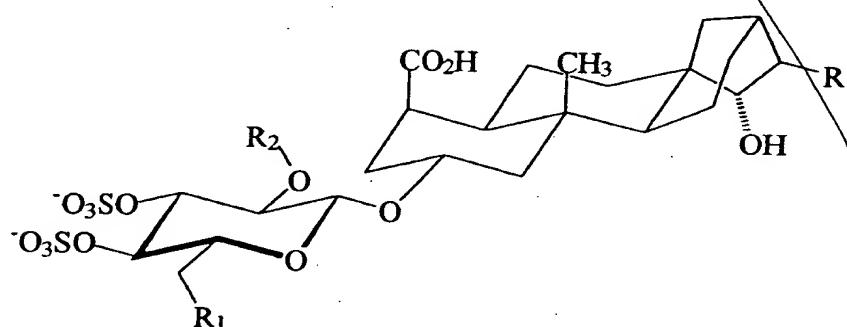
90. The ANT ligand of claim 89 further comprising a  $\text{Eu}^{3+}$  atom complexed to the atractyloside derivative.

91. The ANT ligand of claim 86 wherein the detectable atractyloside derivative comprises covalently bound biotin.

92. The ANT ligand of claim 85 wherein the atractyloside molecule is substituted at 6' hydroxyl with an amine or an amine containing functionality to form an amine modified atractyloside derivative.

93. The ANT ligand according to any one of claims 85-92 wherein the atractyloside molecule is a carboxyatractyloside molecule that is substituted at 6' hydroxyl to form an atractyloside derivative that is a carboxyatractyloside derivative.

94. An ANT ligand having the following structure:



and stereoisomers and pharmaceutically acceptable salts thereof,  
wherein

R<sub>1</sub> is hydroxyl, halogen, -OC(=O)R<sub>4</sub> or -NHR<sub>4</sub>;

R<sub>2</sub> is hydrogen or -C(=O)R<sub>5</sub>;

R<sub>3</sub> is -CH<sub>3</sub> or =CH<sub>2</sub>;

R<sub>4</sub> is -X-aryl, -X-substituted aryl, -X-arylalkyl, -X-substituted arylalkyl, X-heteroaryl, or -X-heteroarylalkyl, wherein X is an optional amido or alkylamido linker moiety; and

R<sub>5</sub> is alkyl.

95. The ANT ligand of claim 94 wherein R<sub>1</sub> is hydroxyl.

96. The ANT ligand of claim 94 wherein R<sub>1</sub> is -C(=O)R<sub>4</sub>.

97. The ANT ligand of claim 94 wherein R<sub>1</sub> is -NHR<sub>4</sub>.

98. The ANT ligand of claim 94 wherein R<sub>2</sub> is hydrogen.

99. The ANT ligand of claim 94 wherein R<sub>2</sub> is -C(=O)R<sub>5</sub>.

100. The ANT ligand of claim 94 wherein R<sub>3</sub> is -CH<sub>3</sub>.

101. The ANT ligand of claim 94 wherein R<sub>3</sub> is =CH<sub>2</sub>.

102. The ANT ligand of claim 94 wherein R<sub>4</sub> is -X-aryl, -X-substituted aryl, -X-arylalkyl or -X-substituted arylalkyl.

103. The ANT ligand of claim 95 wherein R<sub>5</sub> is -CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>.

104. An assay plate for high throughput screening of candidate agents that bind to at least one ANT polypeptide, comprising:

an assay plate having a plurality of wells, each of said wells further comprising at least one immobilized recombinant ANT polypeptide or a variant or fragment thereof.

105. A method of targeting a polypeptide of interest to a mitochondrial membrane, comprising:

expressing a recombinant expression construct encoding a fusion protein in a host cell, said construct comprising at least one regulated promoter operably linked to a nucleic acid molecule comprising a first nucleic acid sequence and a second nucleic acid sequence, said first nucleic acid sequence encoding an adenine nucleotide translocator polypeptide that is expressed as a fusion protein with a polypeptide product of said second nucleic acid sequence, wherein said second nucleic acid sequence encodes the polypeptide of interest.

106. A method of targeting a polypeptide of interest to a mitochondrial membrane, comprising:

expressing a recombinant expression construct encoding a fusion protein in a host cell, said construct comprising at least one promoter operably linked to a nucleic acid molecule comprising a first nucleic acid sequence and a second nucleic acid sequence, said first nucleic acid sequence encoding an animal adenosine nucleotide translocator polypeptide that is expressed as a fusion protein with a polypeptide product of said second nucleic acid sequence, wherein said second nucleic acid sequence encodes the polypeptide of interest.

107. A pharmaceutical composition comprising an ANT ligand of claim 85.

108. A pharmaceutical composition comprising an ANT ligand of claim 94.
109. A pharmaceutical composition comprising an agent that binds to an ANT polypeptide identified according to claim 75.
110. A pharmaceutical composition comprising an agent that binds to an ANT polypeptide identified according to claim 83.
111. A pharmaceutical composition comprising an agent that interacts with an ANT polypeptide identified according to claim 84.
112. A method of treatment comprising administering to a subject the pharmaceutical composition of any one of claims 107-111.